BINDING OF MONOCLONAL ANTI-DNA TO HEPARAN SULPHATE (HS), GBM-HEPARAN SULPHATE PROTEOGLYCAN (GBM-HSPG) AND TO ISOLATED GBM-LOOPS. Jo H.M. Berden*, Rose-Marie Termaat*, Ruud J.T. Smeenk*, Peter Haaber*, Karel J.M. Assmann*. (Intr.by Robert C. Koene) Depts. of Nephrology and Pathology, Univ.Hospital Nijmegen, Netherlands

Previously we have shown (JCI 1986;77:1824) that polyclonal anti-DNA can crossreact with HS in ELISA. Forty-two monoclonal anti-DNA antibodies (MoAb) were obtained by fusion of spleen cells from MRL/l, (NZBxW)F1, and GvH mice. Sixteen of the MoAb crossreacted in ELISA with HS. This binding to HS could be inhibited by DNA. Fifty percent of HS-crossreactive MoAb bound to human GBM-HSPG in ELISA and/or Western blots, but not to HSPG-core protein after removal of HS. Subsequently we isolated GBM loops (GBM-L) from human and rat glomeruli. Ultrastructurally we found a strong binding of cationic ferritin (CaF), indicating that anionic sites were well preserved. With indirect immunofluorescence on cryostat sections of these GBM-L, 7 of the 42 MoAb showed a fine granular staining along the GBM. Binding to GBM-HSPG in ELISA and to GBM-L could be inhibited by DNA. Heparitinase treatment of GBM-L diminished but did not completely prevent binding of either CaF or MoAb. Preincubation of GBM-L with CaF almost completely inhibited the subsequent binding of MoAb. Some MoAb showed a positive GBM-L staining although they did not bind in ELISA to HS or GBM-HSPG. These results demonstrate that monoclonal anti-DNA antibodies can bind directly to HS and to other not yet identified anionic sites in the GBM. The findings suggest that direct binding of anti-DNA to GBM might play a role in the initiation of SLE-nephritis.

TRANSFORMING GROWTH FACTOR β (TGFβ) UNIQUELY REGULATES PRODUCTION AND STRUCTURE OF GLOMERULAR EXTRACELLULAR MATRIX PROTEOGLYCANS. W. Border, S. Okuda*, L. Languino*, E. Ruoslahti*. University of Utah Health Sciences Center, Salt Lake City, UT and La Jolla Cancer Research Foundation, La Jolla, CA.

Accumulation of extracellular matrix (ECM) is a prominent feature of progressive glomerulonephritis. Since some growth factors are known to stimulate ECM production we examined the effects of TGFβ, interleukin-1 (IL-1), platelet-derived growth factor (PDGF) and tumor necrosis factor (TNF) on the production of ECM by rat mesangial cells in culture. Cells were metabolically labeled with ^{35}S sulfate or ^{35}S methionine and conditioned media were analyzed by SDS-PAGE with fluorography combined with the use of enzymes or antibodies for specific molecular identification. In control experiments mesangial cells produced two species of proteoglycan identified as broad bands centered at 200 and 120 KD. These bands correspond in size to the small chondroitin/dermatan sulfate proteoglycans PG I and PG II (decorin) respectively: and, enzyme digestion showed both bands to be composed of chondroitin/dermatan sulfate. Exposure to TGFβ for 48 h greatly increased the PG I band and induced a structural change detected as a shift in electrophoretic mobility. TGFβ also produced a small increase in fibronectin but not laminin or type IV collagen. IL-1, PDGF or TNF had no substantial effects. These experiments show that TGFβ is unique among growth factors in its metabolic effects on glomerular ECM. The release of a substance like TGFβ in glomerulonephritis could stimulate the expansion of ECM and mediate the progression to glomerulosclerosis.